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(54) Method and apparatus for reduction of bias in amperometric sensors

Verfahren und Vorrichtung zur Verringerung asymmetrischer Fehler in amperometrischen Sensoren

Procédé et dispositif pour la réduction des erreurs asymétriques dans des capteurs ampèremétriques

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EP-A- 0 342 820 EP-A- 0 643 300
WO-A-91/09139 US-A- 5 112 455

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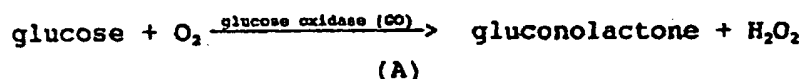
Description

[0001] The present invention generally relates to a biosensor, and, more particularly, to a new and improved method and apparatus for reducing bias in amperometric sensors.

Background of the Invention

[0002] The quantitative determination of analytes in body fluids is of great importance in the diagnoses and maintenance of certain physiological abnormalities. For example lactate, cholesterol and bilirubin should be monitored in certain individuals. In particular, the determination of glucose in body fluids is of great importance to diabetic individuals who must frequently check the level of glucose in their body fluids as a means of regulating the glucose intake in their diets. While the remainder of the disclosure herein will be directed towards the determination of glucose, it is to be understood that the procedure and apparatus of this invention can be used for the determination of other analytes upon selection of the appropriate enzyme. The ideal diagnostic device for the detection of glucose in fluids must be simple, so as not to require a high degree of technical skill on the part of the technician administering the test. In many cases, these tests are administered by the patient which lends further emphasis to the need for a test which is easy to carry out. Additionally, such a device should be based upon elements which are sufficiently stable to meet situations of prolonged storage.

[0003] Methods for determining analyte concentration in fluids can be based on the electrochemical reaction between the analyte and an enzyme specific to the analyte and a mediator which maintains the enzyme in its initial oxidation state. Suitable redox enzymes include oxidases, dehydrogenases, catalase and peroxidase. For example, in the case where glucose is the analyte, the reaction with glucose oxidase and oxygen is represented by equation (A).



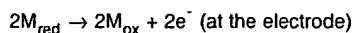
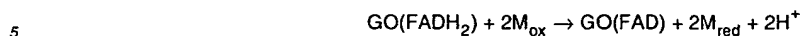
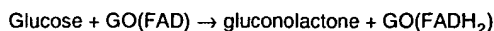
[0004] In a colorimetric assay, the released hydrogen peroxide, in the presence of a peroxidase, causes a color change in a redox indicator which color change is proportional to the level of glucose in the test fluid. While colorimetric tests can be made semi-quantitative by the use of color charts for comparison of the color change of the redox indicator with the color change obtained using test fluids of known glucose concentration, and can be rendered more highly quantitative by reading the result with a spectrophotometric instrument, the results are generally not as accurate nor are they obtained as quickly as those obtained using a biosensor. As used herein, the term biosensor is intended to refer to an analytical device that responds selectively to analytes in an appropriate sample and converts their concentration into an electrical signal via a combination of a biological recognition signal and a physico-chemical transducer. Aside from its greater accuracy, a biosensor is an instrument which generates an electrical signal directly thereby facilitating a simplified design. In principle, all the biosensor needs to do is measure the time and read the current. Furthermore, a biosensor offers the advantage of low material cost since a thin layer of chemicals is deposited on the electrodes and little material is wasted.

[0005] Referring to the above equation (A), a suitable electrode can measure the formation H_2O_2 due to its introduction of electrons into the test fluid according to equation B:



The electron flow is then converted to the electrical signal which directly correlates to the glucose concentration.

[0006] In the initial step of the reaction represented by equation (A), glucose present in the test sample converts the oxidized flavin adenine dinucleotide (FAD) center of the enzyme into its reduced form, (FADH_2). Because these redox centers are essentially electrically insulated within the enzyme molecule, direct electron transfer to the surface of a conventional electrode does not occur to any measurable degree in the absence of an unacceptably high cell voltage. An improvement to this system involves the use of a nonphysiological redox coupling between the electrode and the enzyme to shuttle electrons between the (FADH_2) and the electrode. This is represented by the following scheme in which the redox coupler, typically referred to as a mediator, is represented by M:



[0007] In the scheme, GO(FAD) represents the oxidized form of glucose oxidase and GO(FADH₂) indicates its reduced form. The mediating species M_{ox}/M_{red} shuttles electrons from the reduced enzyme to the electrode thereby oxidizing the enzyme causing its regeneration in situ which, of course, is desirable for reasons of economy. The main purpose for using a mediator is to reduce the working potential of the sensor. An ideal mediator would be re-oxidized at the electrode at a low potential under which impurity in the chemical layer and interfering substances in the sample would not be oxidized thereby minimizing interference.

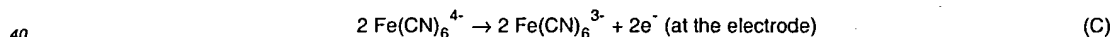
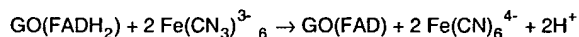
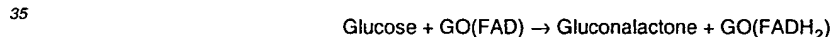
[0008] Many compounds are useful as mediators due to their ability to accept electrons from the reduced enzyme and transfer them to the electrode. Among the mediators known to be useful as electron transfer agents in analytical determinations are the substituted benzo- and naphthoquinones disclosed in U.S. Patent 4,746,607; the N-oxides, nitroso compounds, hydroxylamines and oxines specifically disclosed in EP 0 354 441; the flavins, phenazines, phenothiazines, indophenols, substituted 1,4-benzoquinones and indamins disclosed in EP 0 330 517 and the phenazinium/phenoxazinium salts described in U.S. Patent 3,791,988. A comprehensive review of electrochemical mediators of biological redox systems can be found in *Analytica Clinica Acta*, 140 (1982), Pp 1-18.

[0009] Among the more venerable mediators is hexacyanoferrate, also known as ferricyanide, which is discussed by Schl pfer et al in *Clinica Chimica Acta*, 57 (1974), Pp. 283-289. In U.S. Patent 4,929,545 there is disclosed the use of a soluble ferricyanide compound in combination with a soluble ferric compound in a composition for enzymatically determining an analyte in a sample. Substituting the iron salt of ferricyanide for oxygen in equation (A) provides:



since the ferricyanide is reduced to ferrocyanide by its acceptance of electrons from the glucose oxidase enzyme.

[0010] Another way of expressing this reaction is by use of the following equation (C):



[0011] The electrons released are directly equivalent to the amount of glucose in the test fluid and can be related thereto by measurement of the current which is produced through the fluid upon the application of a potential thereto. Oxidation of the ferrocyanide at the anode renews the cycle.

[0012] As is apparent from the above description, a necessary attribute of a mediator is the ability to remain in the oxidized state under the conditions present on the electrode surface prior to the use of the sensor. Any reduction of the mediator will increase the background current resulting in the biosensor reading being biased. It has been discovered that these mediators do tend to be reduced over time, especially under conditions of stress, thereby diminishing the usefulness of the sensors to which they are applied.

[0013] In published international patent application WO 92/15704 there is disclosed the use of potassium dichromate as an oxidizing agent in a colorimetric reagent strip. The purpose of the oxidizing agent is to oxidize impurities in other reagent components to improve the colorimetric sensor's stability. This publication mentions USSN 07/451,671 (now US 5,288,636) and characterizes it as describing a system in which a reduced mediator is re-oxidized by the application of a potential and measuring the current after a specific time to determine the concentration of the analyte. More specifically, the '636 patent requires the complete oxidation of the glucose by glucose oxidase. As the enzyme is reduced by the glucose, the ferricyanide reacts with enzyme to produce ferrocyanide. The ferrocyanide produced by this enzymatic reaction is combined with ferrocyanide produced during storage. This latter ferrocyanide is the result of a reaction between ferricyanide and impurities found in materials deposited with the glucose oxidase and ferricyanide.

The '636 patent makes no distinction between ferrocyanide produced between these two sources.

[0014] EP-A-0 342 820 discloses a method for measuring the concentration of a substance in a sample solution which comprises (i) preparing a test solution having the sample and an excess of redox mediator (ii) presenting the test solution to first electrode means to ensure that the redox mediator is completely oxidized, (iii) passing the test solution through an enzyme channel, whereby a quantity of redox mediator is reduced, and (iv) passing the test solution to second electrode means to re-oxidize said quantity of redox mediator, whereby the charge corresponding to this process is measured.

[0015] WO-A-91/09139 discloses an amperometric biosensor which comprises a working and a counter electrodes that are partially covered with a reagent having a sufficient amount of redox mediator and an enzyme. The concentration of analyte in a sample is detected with this biosensor by (i) adding the sample solution to the reagent, (ii) letting react (oxidize/reduce) the analyte with the enzyme completely, whereby an amount of redox mediator is reduced/oxidized, and (iii) applying a potential difference between both electrodes such that the mediator is electrore-oxidized/reduced and measuring the current produced during the process.

[0016] It would be desirable, and it is an object of the present invention to provide a method whereby the undesired reduction of mediator compounds stored on an electrodes surface can be reversed to minimize its effect on estimating the analyte values in fluid test samples with very low analyte concentrations.

[0017] It is a further object to provide such a method in which the accuracy of the analyte determination is enhanced.

[0018] It is a further object to provide such a method wherein the analyte is glucose.

[0019] An additional object is to provide a mathematical means for further enhancement of the accuracy of the analyte determination.

[0020] It is a further object to provide apparatus for accurately determining analyte values.

[0021] It is further object to provide such apparatus that is simple and economical to manufacture.

Summary of the Invention

[0022] The present invention involves a method for determining the concentration of an analyte in a fluid test sample by applying the test sample to the surface of a working electrode. The electrode has on its surface a composition comprising an enzyme specific for the analyte, a mediator which is reduced as a result of a reaction between the analyte and the enzyme, which mediator has undergone partial reduction to its reduced state as a result of having been exposed to ambient conditions. There is disclosed herein an improvement to the method which involves the steps of:

a) applying a positive potential pulse to the electrode to oxidize at least a portion of the mediator to its oxidized form. This step reduces background bias in the electrode. The background bias can be further reduced by:

a) determining the current (i_1) during the application of the positive pulse and the current (i_2) at the end of the read time, and

b) calculating the corrected analyte level G by solving equation (1):

$$G = \frac{i_2 - \text{Int}}{\text{slope}} - k \cdot \Delta(i_1, i_2) \quad (1)$$

where Int and slope are the intercept and slope of i_2 and $\Delta(i_1, i_2)$ is an error correction term proportional to the background bias calculated as:

$$\Delta(i_1, i_2) = \frac{\text{Int}}{\text{slope}} \cdot \left(\frac{\text{slope} \cdot i_1 - s_1 \cdot i_2}{\text{slope} \cdot i_{1,lo} - s_1 \cdot i_{2,lo}} - 1 \right) \quad (2)$$

where

s_1 = slope of i_1

$i_{1,lo}$ = i_1 at a low analyte level,

$i_{2,lo}$ = i_2 at a low analyte level, and

k = a selected scaling factor.

Brief Description of the Drawings

[0023] The present invention together with the above and other objects and advantages may best be understood from the following detailed description of the preferred embodiments of the invention illustrated in the drawings, wherein:

FIG. 1 is a chart illustrating potential and current relative to time in accordance with the method of the invention;

FIG. 2 is a block diagram representation of a device for determining analyte values employed to perform the method of the invention; and

FIG. 3 is a flow chart illustrating the sequential steps performed by a processor of FIG. 2 in accordance with the method of the invention.

Description of the Invention

[0024] The present invention is a method that reduces the background bias due to oxidizable impurities in an amperometric sensor used for measuring a specific analyte, such as glucose, in blood. The background current of such a sensor will increase if it is stored over a long period of time or under stress (heat, moisture, etc.) due to the increased presence of reduced mediator or other reduced impurity present in the sensor such as enzyme stabilizers, e.g. glutamate, and surfactants having reducing equivalents. For example, in a ferricyanide based amperometric sensor, the background bias is related to the presence of ferrocyanide (from the reduction of ferricyanide) near the electrode surface. This accumulated ferrocyanide, as opposed to the ferrocyanide produced during use of the sensor (fresh ferrocyanide), is oxidized back to ferricyanide to reduce the background bias it causes and thereby extend the sensor shelf life. To achieve this objective, the method uses an electrochemical approach. The background bias is further reduced when the electrochemical approach is augmented with an algorithmic correction.

[0025] Referring to FIG. 1, the method of our invention involves first applying a positive potential pulse (called the "burn-off" pulse) which precedes the normal potential profile during use of the sensor. This is typically accomplished by applying a positive potential of from 0.1 to 0.9 volt (preferably 0.3 to 0.7 volt) between the working and reference electrodes of the sensor for a period of from 1 to 15 seconds (preferably 5 to 10 seconds). The burn-off pulse oxidizes the initial ferrocyanide (or other oxidizable impurity), so that the sensor can begin the assay with a clean background. Typically, the background is not perfectly clean since only a portion of the oxidizable impurity is oxidized by the burn-off pulse. This is the case because the chemical layer covers both the working and the reference electrodes. The initial ferrocyanide exists in the chemical layer since it comes from ferricyanide. When sample fluid is applied and the chemical layer re-hydrates, the ferrocyanide near the working electrode is re-oxidized. The rest of the ferrocyanide diffuses into the sample fluid and is mixed with the glucose. That portion of the initial ferrocyanide cannot be re-oxidized without affecting the glucose. The initial ferrocyanide is near the electrode for a very short time (a few seconds) after the fluid test sample is applied. The reason for this is that the chemicals (enzyme and ferricyanide, etc.) are deposited as a thin layer on the working and reference electrodes. The burn-off technique takes advantage of this since a significant amount of the initial ferrocyanide can be burned off without noticeable reduction of the analyte concentration in the fluid test sample most of which does not come into direct contact with the electrode. Experiments have demonstrated that the background bias of a stressed sensor can be reduced by 40% with proper application of the burn-off pulse.

[0026] The background bias can be further reduced by the use of a background correction algorithm which works in conjunction with the burn-off pulse. The algorithm is based on the taking of two current readings. The first reading (i_1) is taken during the burn-off pulse and the second (i_2) at the end of the read time, i.e. the time elapsed from the moment when the second potential pulse is applied to the moment when the current i_2 is measured. The length of the read time is $t_3 - t_2$, as shown in FIG. 1. The analyte concentration is then calculated from the two current readings, i_1 and i_2 . Tests on sensors have shown that the background correction algorithm is able to remove at least 80% of the remaining background bias, and, as a result, the sensor stability can be improved to provide a significant extension in shelf life.

[0027] An amperometric glucose sensor of the type useful in the practice of the present invention is constructed as follows: Two carbon electrodes are printed on a polymer substrate. Next a layer of chemical components is deposited on the electrodes and dried. A preferred chemical composition is 5 μ L of a medium containing 55 mM ferricyanide (potassium salt), 8.5 units of glucose oxidase, 0.53% of poly(ethylene oxide), 0.40% of cremophor as surfactant and 83 mM phosphate buffer at pH 7.2. During the glucose assay, a potential profile consisting of three consecutive time periods is applied to the sensor. These time periods are, in sequence, the burn-off time (typically 0.4 volt for 10 seconds); delay period (open circuitry for 15 seconds) and read time (0.4 volts, 5 seconds). The exact time of the delay period is not critical but is normally in the range of 10 to 40 seconds. This delay period allows sufficient time for the reaction to build up sufficient ferrocyanide to allow the current resulting from the reoxidation of the ferrocyanide to be measured without difficulty. These time periods are illustrated in FIG. 1 which plots potential and current against time. Current

measurements are taken at the end of the burn-off period (i_1) and read time (i_2) whereupon the corresponding glucose concentration is calculated using equation 1. The constants in the equation, e.g. slopes and intercepts are predetermined values.

[0028] The following discussion relates to a fluid test sample in which glucose is the analyte to be detected and involves a sensor in which ferricyanide is the mediator. However, the discussion is equally applicable to systems for the determination of other analytes and in which the oxidizable species is something other than ferrocyanide.

[0029] The burn-off technique, i.e. application of a positive potential pulse to the electrode to oxidize at least a portion of the mediator back to its oxidized form, is illustrated by FIG. 1. In FIG. 1, in which the potential and current profiles are plotted, the timing is as follows:

t_0 - sample is detected, burnoff period begins. Sample is detected by inserting the sensor into the instrument which causes the immediate application of a 0.4 volt potential. The current is continuously checked to see if a larger than predetermined threshold (e.g. 250 nA) is measured. When a larger current than the threshold value is detected, a sample has been detected to begin the burnoff time period.

t_1 - end of burn-off period and current i_1 is measured. The length of the burnoff period, $t_1 - t_0$, is usually 5 to 10 seconds. The potential is 0.4 volt at t_1 but switches to an open circuit or to a potential that substantially reduces the current to minimize the rate of electrochemical reaction at the working electrode for a set delay period after the burnoff period.

t_2 - end of set delay period. The length of the wait period, $t_2 - t_1$, is normally 10 to 40 seconds. A read potential of 0.4 volt is applied at t_2 .

t_3 - end of read time when current i_2 is measured. The length of the read time, $t_3 - t_2$, is 5 to 10 seconds.

[0030] The burn-off pulse, i.e. application of the 0.4 volt potential from t_0 to t_1 , is designed to eliminate part of initial ferrocyanide (accumulated ferro) or other oxidizable interferences in the enzyme layer.

[0031] The burn-off algorithm calculates glucose concentration from two current measurements i_1 and i_2 using equation 1:

$$G = \frac{i_2 - \text{int}}{\text{slope}} K \cdot \Delta(i_1, i_2) \quad (1)$$

where

$$\Delta(i_1, i_2) = \frac{\text{int}}{\text{slope}} \cdot \left(\frac{\text{slope} \cdot i_1 - s_1 \cdot i_2}{\text{slope} \cdot i_{1,0} - s_1 \cdot i_{2,0}} - 1 \right) \quad (2)$$

[0032] Equation 1 is a partial correction algorithm which is intended to achieve a compromise between reducing stress-related background bias and preserving system precision. The basic scheme is to use i_2 as a glucose reading

$$G = \frac{i_2 - \text{int}}{\text{slope}}$$

where int and slope are the intercept and slope of i_2 respectively. The term $\Delta(i_1, i_2)$ is the estimated background increase, due to stress or other causes, derived from the current i_1 and i_2 . For fresh sensors, this term is close to zero. The parameter k is selectively provided or set to a value from 0 to 1. There will be no background correction if k is set at zero. On the other hand a full correction can be achieved if k is 1. In the following examples k is set at 0.8 for partial correction because it has been found that the variation of i_1 is larger than that of i_2 when multiple sensors are tested under the same glucose concentration. Compared with the glucose value calculated from i_2 alone, $k = 0$ in equation (1), the glucose value calculated from i_1 and i_2 jointly will be slightly lower in precision (a larger standard deviation) and, of course, a much smaller background bias. The tradeoff between the precision and bias can be achieved by choosing the proper k value. If $k = 0$, there is no background correction and i_1 is not used. In this case, the highest

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precision can be obtained, but it is accompanied by a high background bias. If $k = 1$, the full background correction is applied whereupon the lowest bias can be achieved but at the cost of precision. The k value is set at 0.8 in the example to achieve a compromise between precision and bias.

[0033] The parameters in these equations are:

Int - intercept of read current i_2, nA .

$slope$ - slope of read current $i_2, nA \cdot dL/mg$.

i_{1_lo} - average burn-off current i_1, nA , at the low glucose calibration level, i.e. 50 mg/dL .

i_{2_lo} - average read time current i_2, nA , at the low glucose calibration level. Actually, i_{2_lo} is not an independent parameter. It can be calculated from Int and $slope$:

$$i_{2_lo} = Int + slope \cdot 50.$$

S_1 - slope to burn-off current, $nA \cdot dL/mg$.

k - set to 0.8 for partial correction.

[0034] Int , $slope$, i_{1_lo} , and s_1 are local parameters; each sensor lot has its own parameter values which values are determined experimentally. The algorithm needs two known current values, one for i_1 and one for i_2 for normal (unstressed) sensors. The i_{1_lo} and i_{2_lo} are available since they are used in determining the intercept (Int) and slopes (s_1 and $slope$). Of course, current at other glucose levels can be used in the algorithm. This would however introduce the extra step of adding two additional independent parameters. The procedure of the present invention is demonstrated in the following examples:

Example I:

[0035] The following steps are taken to determine the lot parameter values necessary in the algorithm:

A. Test 16 sensors from the lot at the low calibration level, 50 mg/dL , and obtain the average currents i_{1_lo} and i_{2_lo} of the burn-off current and read time current, respectively. It is found that $i_{1_lo} = 1951.2 \text{ nA}$ and $i_{2_lo} = 1952.3 \text{ nA}$.

B. Test 16 sensors at the high calibration level, 400 mg/dL , and obtain the average current i_{1_hi} and i_{2_hi} . It is found that $i_{1_hi} = 6003.3 \text{ nA}$ and $i_{2_hi} = 8831.7 \text{ nA}$.

C. Calculate the parameter values:

$$Int = i_{2_lo} - \frac{50 \cdot (i_{2_hi} - i_{2_lo})}{350} = 1952.3 - \frac{50 \cdot (8831.7 - 1952.3)}{350} = 969.5 \text{ nA}$$

$$slope = \frac{i_{2_hi} - i_{2_lo}}{350} = \frac{8831.7 - 1952.3}{350} = 19.65 \text{ nA} \cdot dL/mg$$

$$s_1 = \frac{i_{1_hi} - i_{1_lo}}{350} = \frac{6003.3 - 1951.2}{350} = 11.58 \text{ nA} \cdot dL/mg$$

[0036] Therefore, equation (1) becomes:

$$G = \frac{i_2 - 969.5}{19.65} - 0.8 \cdot \Delta(i_1, i_2)$$

$$\Delta(i_1, i_2) = \frac{969.5}{19.65} \cdot \left(\frac{19.65 \cdot i_1 - 1158 \cdot i_2}{19.65 \cdot 19512 - 1158 \cdot 19523} - 1 \right)$$

$$= 0.06162 \cdot i_1 - 0.03631 \cdot i_2 - 49.34$$

Example II

[0037] It has been discovered that the burn-off pulse alone will significantly reduce the background bias even without the use of the background correction algorithm.

[0038] In this experiment, ten sensors were stressed under 30°C and 91% humidity for 3 hours. Aqueous glucose at 50 mg/dL was used as sample. Five stressed sensors were tested with a 10 second burn-off pulse and five without the pulse. In addition, ten unstressed sensors were tested as control (five with the 10 second burn-off and five without) and the bias calculated using the following equation (3):

$$\text{bias} = \frac{i_{\text{stressed}} - i_{\text{unstressed}}}{i_{\text{unstressed}}} \times 100\% \quad (3)$$

It was found that the bias was 30.6% without the burn-off pulse and 18.0% with it which data demonstrate that the burn-off pulse alone reduces the background bias by about 40%.

Example III

[0039] This example explains how the algorithm corrects for background bias:

[0040] Eight sensors were stored at below -20°C for two weeks and another eight sensors were stressed at 50°C for four weeks. All sixteen sensors were tested using whole blood having a 100 mg/dL glucose concentration. The parameter values were determined from fresh sensors. The glucose readings, G, were calculated as follows:

A. No background bias correction algorithm: Equation 1 with k = 0.

B. Partial correction: Equation 1 with k = 0.8.

[0041] The bias in percent is calculated using Equation 4 with the results being listed in Table 1.

$$\text{bias} = \frac{G - 100}{100} \times 100\% \quad (4)$$

Table 1

Bias at 100 mg/dL		
	no burn-off algorithm	partial correction (k = 0.8)
-20°C. 2 weeks	3.8 %	5.3 %
50°C. 4 weeks	64.7 %	15.0 %

[0042] A device capable of carrying out the invention is represented by FIG. 2. Referring to FIG. 2, there is shown a block diagram representation of a device for accurately determining analyte values designated as a whole by the reference character 10 and arranged in accordance with principles of the present invention. Device 10 includes a microprocessor 12 together with a memory device 14. Microprocessor 12 is suitably programmed to perform the method of the invention as illustrated in FIG. 3. Various commercially available devices, such as a DS5000 microcontroller manufactured by Dallas Semiconductor, can be used for the microprocessor 12 and memory 14. Memory 14 can be included within the microprocessor 12 or separately provided as illustrated in FIG. 2.

[0043] Digital data from the microprocessor 12 is applied to a digital-to-analog (D/A) converter 16. D/A converter 16 converts the digital data to an analog signal. An amplifier 18 coupled to the D/A converter 16 amplifies the analog signal. The amplified analog signal output of amplifier 18 is applied to a sensor 20.

[0044] Sensor 20 is coupled to an amplifier 22. The amplified sensed signal is applied to an analog-to-digital (A/D) converter 24 that converts the amplified, analog sensor signal to a digital signal. The digital signal is applied to the microprocessor 12.

[0045] Various commercially available devices can be used for D/A converter 16, amplifiers 18 and 20 and A/D converter 24. For example, a device type PM-752F4FS manufactured by PMI can be used for D/A converter 16. Operational amplifier device type TL074AC manufactured and sold by Linear Technology can be used for amplifiers 18 and 22. A device type MAX 135 CWI manufactured and sold by Maxum can be used for the A/D converter 24.

[0046] Referring also to FIG. 3, there are shown the sequential steps for accurate analyte determination of the invention. Initially microprocessor 12 applies a burnoff pulse, for example a potential of 0.4 volts, to the sensor 20 as indicated at a block 300. Then the microprocessor checks to identify a sample corresponding to a detected sensor threshold current value as indicated at a decision block 302. When a sample is detected at block 302, a predetermined burnoff time interval, such as 10 seconds is identified at a decision block 304. Next the current i_1 is measured as indicated at a block 306 and an open circuit is applied to the sensor 20 as indicated at a block 308. Then a set delay or predetermined wait time interval, such as fifteen (15) seconds is identified at a decision block 310. After the set delay, a read pulse or potential of 0.4 volts is applied to the sensor 20 as indicated at a block 312. Then a predetermined read time interval for the read pulse, such as 5 seconds is identified at a decision block 314 and the current i_2 is measured as indicated at a block 316. Next microprocessor 12 gets the stored parameters for a particular sensor 20 including I_{nt} , slope, $i_{1,10}$, $i_{2,10}$, S_1 and k , as indicated at a block 320. The correction term Delta (i_1 , i_2) is calculated utilizing the stored parameters and measured burn-off current i_1 and read current i_2 as indicated block 322. Next the analyte value, such as glucose reading G , is calculated utilizing the read current i_2 and the calculated correction term Delta (i_1 , i_2) multiplied by the selected scaling value k , as indicated at a block 324.

Claims

1. Method to increase the accuracy of the determination of the concentration of an analyte in a fluid test sample by applying the fluid test sample to the surface of a working electrode

which is electrochemically connected to a reference electrode

which surface bears a composition comprising

an enzyme specific for the analyte,

a mediator which is reduced in response to a reaction between the analyte and the enzyme, which mediator has undergone partial reduction and then

determining the concentration of the analyte in the fluid test sample as a function of the current which passes through the fluid test sample

by measuring the current between the working and reference electrodes

which current results from the amount of reduced mediator produced

during storage of the sensor at ambient conditions and

during the time period prior to the current measurement period,

characterized in that

a.) a first potential is applied between the electrodes to return at least a portion of the mediator back to its oxidized form and

b.) the system is switched to an open circuit or to a potential that reduces the current to minimize the rate of electrochemical reaction at the working electrode for a set delay period and

c.) a second potential is applied between the working and the reference electrode to oxidize the reduced mediator at a time interval which is normally in the range of 10 to 40 seconds after applying the potential and

d.) the current at the working electrode is measured.

2. The method of Claim 1 wherein the accuracy of the analyte determination is further increased by:

- 5 a) determining the current (i_1) at the end of the application of the first potential pulse and the current (i_2) at the end of the application of the second potential pulse, and
b) calculating the corrected analyte level G by solving the equation:

$$10 \quad G = \frac{i_2 \cdot \text{Int}}{\text{slope}} K \cdot \Delta(i_1, i_2)$$

where Int is the intercept of the linear function of current i_2 versus the concentration of the analyte and slope is the slope of the linear function of current i_2 versus the concentration of the analyte, K is a parameter of value from 0 to 1 and $\Delta(i_1, i_2)$ is an error correction term proportional to the background bias calculated as:

$$20 \quad \Delta(i_1, i_2) = \frac{\text{Int}}{\text{slope}} \cdot \left(\frac{\text{slope} \cdot i_1 - s_1 \cdot i_2}{\text{slope} \cdot i_{1,lo} - s_1 \cdot i_{2,lo}} - 1 \right)$$

where

25 s_1 = slope of the linear function of current i_1 versus the concentration of the analyte $i_{1,lo} = i_1$ at the low analyte level used to determine s_1 , and
 $i_{2,lo} = i_2$ at the low analyte level used to determine slope.

3. The method of Claim 1 wherein the mediator is a ferricyanide salt.
- 30 4. The method of Claim 2 wherein the analyte is glucose.
5. The method of Claim 1 wherein the step of applying said oxidizing potential between the electrodes includes the steps of applying a voltage potential selectivity provided in a range between 0.1 volts and 0.9 volts, comparing the measured current with a threshold value and identifying a predetermined time interval for applying said voltage potential responsive to a measured current greater than said threshold value.
- 35 6. The method of Claim 5 wherein said step of identifying a predetermined time interval includes identifying a set time interval selectively provided in a range between 1 second and 15 seconds.
- 40 7. The method of Claim 1 wherein said step of providing a selected time delay includes the step of waiting a selected time delay in a range between 10 seconds and 40 seconds before said second potential is applied and wherein said second potential is applied for a selected time period in a range between 5 seconds and 10 seconds before the step of measuring said current.

45 Patentansprüche

1. Verfahren zur Steigerung der Genauigkeit der Bestimmung der Konzentration eines Analyt in einer flüssigen Testprobe, wobei man

50 die flüssige Testprobe auf der Oberfläche einer Arbeitselektrode vorlegt, die elektrochemisch mit einer Bezugselektrode verbunden ist, wobei die Oberfläche eine Zusammensetzung aufweist, umfassend

ein für den Analyt spezifisches Enzym,

55 einen Mediator, der in Reaktion auf eine Reaktion zwischen dem Analyt und dem Enzym reduziert wird und schon einer Teilreduktion unterzogen worden war,

und man dann die Konzentration des Analyt in der flüssigen Testprobe als Funktion der Stromstärke bestimmt, die durch die flüssige Testprobe hindurchgeht,

indem man die Stromstärke zwischen der Arbeits- und Bezugselektrode misst, welche aus der Menge des reduzierten Mediators resultiert, der während der Lagerung des Sensors bei Umgebungsbedingungen und während der Zeitdauer vor der Stromstärkenmessdauer erzeugt worden war,

dadurch gekennzeichnet, dass

- a) ein erstes Potential zwischen den Elektroden angelegt wird, um zumindest einen Teil des Mediators zurück in seine oxidierte Form zu bringen, und
 - b) das System auf einen offenen Stromkreis oder auf ein Potential übertragen wird, das die Stromstärke herabsetzt, um den Umsatz der elektrochemischen Reaktion an der Arbeitselektrode für eine festgelegte Verzugszeitspanne zu minimieren, und
 - c) ein zweites Potential zwischen der Arbeits- und Bezugselektrode angelegt wird, um den reduzierten Mediator bei einem Zeitintervall zu oxidieren, das normalerweise im Bereich von 10 bis 40 s nach Anlegen des Potentials liegt, und
 - d) die Stromstärke an der Arbeitselektrode gemessen wird.
2. Verfahren gemäß Anspruch 1, wobei die Genauigkeit der Analyt-Bestimmung weiter gesteigert wird durch:
- a) Bestimmung der Stromstärke (i_1) am Ende des Anlegens des ersten Potentialstoßes und der Stromstärke (i_2) am Ende des Anlegens des zweiten Potentialstoßes, und
 - b) Berechnung der korrigierten Analyt-Gehaltsmenge G durch Lösen der Gleichung:

$$G = \frac{i_2 \times \text{Int}}{\text{Steigung}} - k \times \Delta(i_1, i_2),$$

worin Int das Interzept (der Abschnitt) der Linearfunktion von Stromstärke (i_2) gegen die Konzentration des Analyt und Steigung die Steigung der Linearfunktion von Stromstärke i_2 gegen die Konzentration des Analyt, k ein Parameter mit einem Wert von 0 bis 1 und $\Delta(i_1, i_2)$ ein Fehlerkorrekturterm proportional zum Hintergrund-Fehler sind, der berechnet wird als:

$$\Delta(i_1, i_2) = \frac{\text{Int}}{\text{Steigung}} \times \left[\frac{\text{Steigung} + i_1 - s_1 \times i_2}{\text{Steigung} \times i_{1_{10}} - s_1 \times i_{2_{10}}} - 1 \right],$$

worin: s_1 = Steigung der Linearfunktion der Stromstärke i_1 gegen die Konzentration des Analyt,
 $i_{1_{10}}$ = i_1 bei niedrigem Analyt-Gehalt zur Bestimmung von s_1 und
 $i_{2_{10}}$ = i_2 bei niedrigem Analyt-Gehalt zur Bestimmung der Steigung.

3. Verfahren gemäß Anspruch 1, wobei der Mediator ein Ferricyanid-Salz ist.
4. Verfahren gemäß Anspruch 2, wobei der Analyt Glucose ist.
5. Verfahren gemäß Anspruch 1, wobei die Stufe des Anlegens des genannten oxidierenden Potentials zwischen den Elektroden die Stufen einschließt, in denen man eine Spannungspotentialselektivität anwendet, die im Bereich von 0,1 bis 0,9 Volt vorgesehen ist, man die gemessene Stromstärke mit einem Schwellenwert vergleicht und ein

vorbestimmtes Zeitintervall zum Anlegen des genannten Spannungspotentials identifiziert, das auf eine gemessene Stromstärke reagiert, die größer als der genannte Schwellenwert ist.

- 5 6. Verfahren gemäß Anspruch 5, wobei die genannte Stufe zur Identifizierung eines vorbestimmten Zeitintervalls einschließt, dass ein festgelegtes Zeitintervall identifiziert wird, das selektiv in einem Bereich von 1 bis 15 s vorgesehen ist.
- 10 7. Verfahren gemäß Anspruch 1, wobei die genannte Stufe zur Vorgabe eines ausgewählten Zeitverzugs die Stufe einschließt, dass ein ausgewählter Zeitverzug in einem Bereich von 10 bis 40 s abgewartet wird, bevor das genannte zweite Potential angelegt wird, und wobei das genannte zweite Potential eine ausgewählte Zeitspanne lang in einem Bereich von 5 bis 10 s angelegt wird, bevor die Stufe zur Messung der genannten Stromstärke durchgeführt wird.

15 Revendications

1. Méthode pour accroître la précision de la détermination de la concentration d'un analyte dans un échantillon d'un fluide d'essai

20 en appliquant l'échantillon de fluide d'essai à la surface d'une électrode de travail qui est connectée électriquement à une électrode de référence surface qui porte une composition comprenant une enzyme spécifique de l'analyte un médiateur qui est réduit en réponse à une réaction entre l'analyte et l'enzyme, médiateur qui a subi une

25 réduction partielle, puis en déterminant la concentration de l'analyte dans l'échantillon de fluide d'essai en fonction du courant qui passe à travers l'échantillon de fluide d'essai en mesurant le courant entre l'électrode de travail et l'électrode de référence courant qui résulte de la quantité de médiateur réduit produite

30 au cours du stockage du capteur dans les conditions ambiantes et au cours de la période de temps avant la période de mesure du courant,

caractérisée en ce que

- 35 a) un premier potentiel est appliqué entre les électrodes pour ramener au moins une partie du médiateur à sa forme oxydée, et b) le système est commuté à un circuit ouvert ou à un potentiel qui réduit le courant pour réduire au minimum la vitesse de réaction électrochimique au niveau de l'électrode de travail pendant une période de retard fixée, et c) un second potentiel est appliqué entre l'électrode de travail et l'électrode de référence pour oxyder le médiateur réduit à un intervalle de temps qui est normalement compris dans l'intervalle de 10 à 40 secondes après application du potentiel, et
- 40 d) le courant au niveau de l'électrode de travail est mesuré.

- 45 2. Méthode suivant la revendication 1, dans laquelle la précision de la détermination de l'analyte est accrue davantage :

- a) en déterminant le courant (i_1) à la fin de l'application de la première impulsion de potentiel et le courant (i_2) à la fin de l'application de la seconde impulsion de potentiel, et b) en calculant la teneur en analyte corrigée G en résolvant l'équation :

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$$G = \frac{i_2 \cdot \text{Int}}{\text{pente}} K \cdot \Delta(i_1, i_2)$$

- 55 dans laquelle Int est l'intercepte de la fonction linéaire du courant i_2 en fonction de la concentration de l'analyte et la pente est la pente de la fonction linéaire du courant i_2 en fonction de la concentration de l'analyte, K est un paramètre ayant une valeur de 0 à 1 et $\Delta(i_1, i_2)$ est un terme de correction d'erreur proportionnel à la tension de polarisation de fond, calculé au moyen de l'équation :

$$\Delta(i_1, i_2) = \frac{\text{Int}}{\text{pente}} \cdot \frac{\text{pente} \cdot i_1 - S_1 \cdot i_2}{\text{pente} \cdot i_{1-10} - S_1 \cdot i_{2-10}} - 1$$

5 dans laquelle

S_1 = pente de la fonction linéaire du courant i_1 en fonction de la concentration de l'analyte

$i_{1-10} = i_1$ à la basse teneur en analyte utilisée pour déterminer S_1 , et

$i_{2-10} = i_2$ à la basse teneur en analyte utilisée pour la détermination de la pente.

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3. Méthode suivant la revendication 1, dans laquelle le médiateur est un sel consistant en un ferricyanure.

4. Méthode suivant la revendication 2, dans laquelle l'analyte est le glucose.

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5. Méthode suivant la revendication 1, dans laquelle l'étape d'application du potentiel d'oxydation entre les électrodes comprend les étapes consistant à appliquer une sélectivité de potentiel de tension comprise dans l'intervalle de 0,1 volt à 0,9 volt, à comparer le courant mesuré à une valeur seuil et identifier un intervalle de temps prédéterminé pour l'application dudit potentiel de tension pouvant répondre à un courant mesuré supérieur à ladite valeur seuil.

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6. Méthode suivant la revendication 5, dans laquelle l'étape d'identification d'un intervalle de temps prédéterminé comprend l'identification d'un intervalle de temps fixé de manière sélective compris dans l'intervalle de 1 seconde à 15 secondes.

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7. Méthode suivant la revendication 1, dans laquelle l'étape d'établissement d'un retard choisi comprend l'étape d'attente d'un retard choisi dans l'intervalle de 10 secondes à 40 secondes avant l'application du second potentiel, et dans laquelle ledit second potentiel est appliqué pendant une période de temps choisie dans l'intervalle de 5 secondes à 10 secondes avant l'étape de mesure dudit courant.

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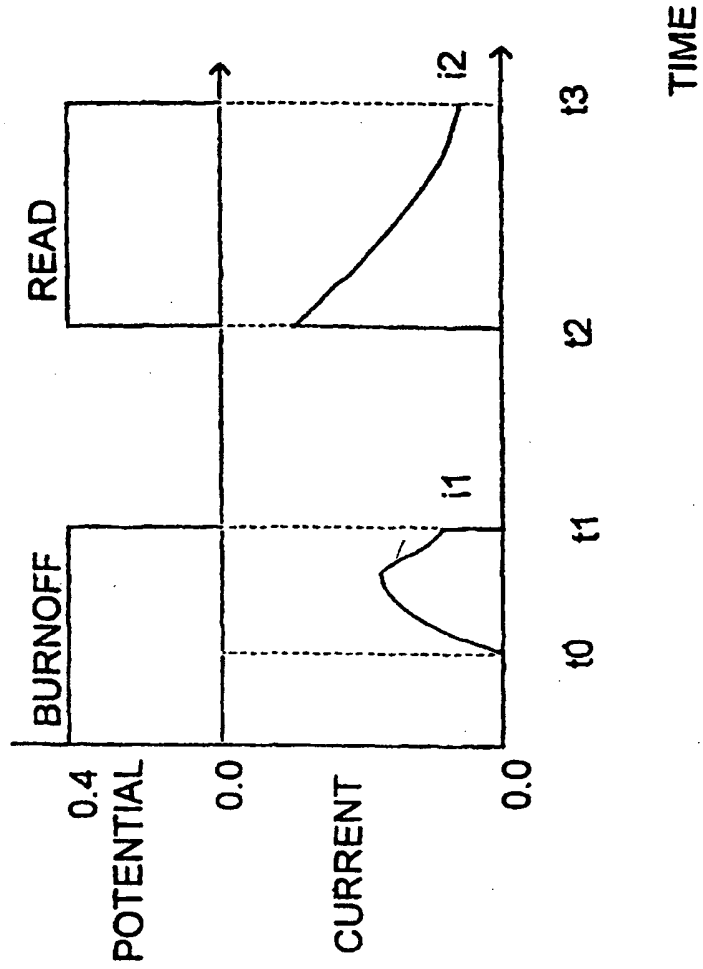


FIG.1

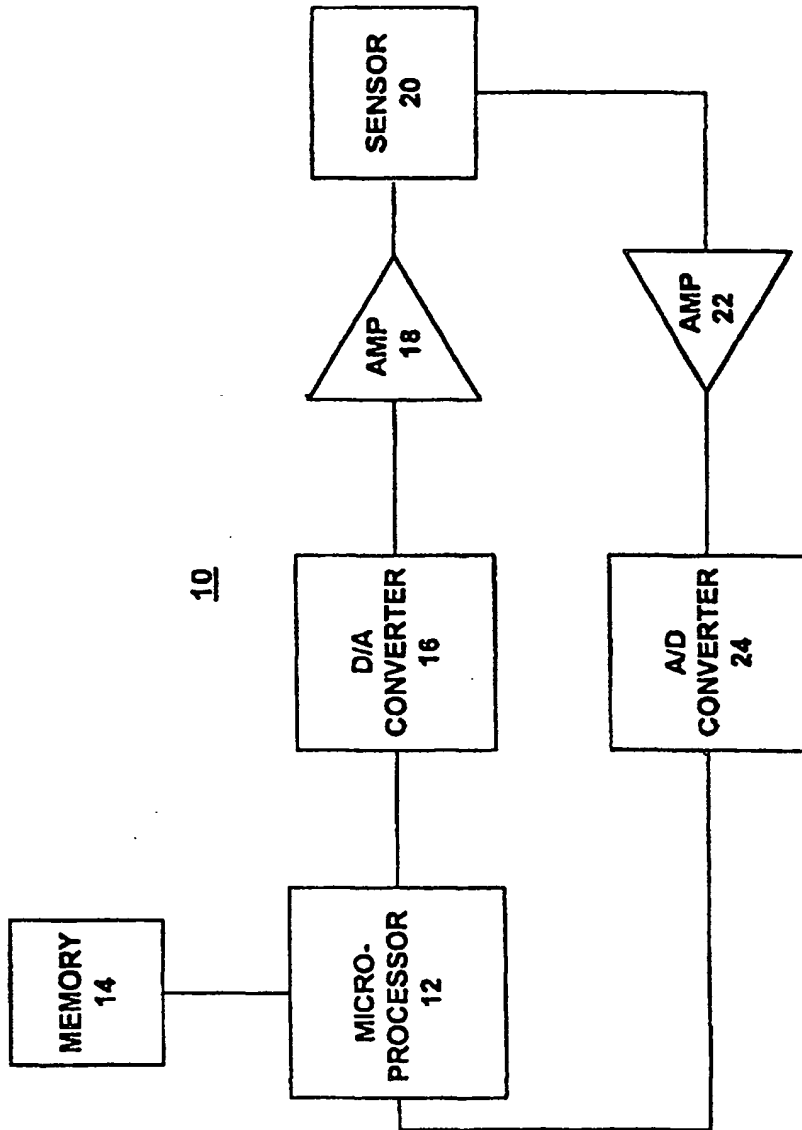


FIG.2

FIG.3

